ASSOCIATION OF INTESTINAL HELMINTHS WITH DECREASED LIVER SIZE AND sCD23 CONCENTRATION DURING FALCIPARUM MALARIA

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Abstract. To determine if intestinal helminths and the CD23/nitric oxide pathway had an influence on liver size, we conducted a cross-sectional study on 438 patients with confirmed *P. falciparum* malaria admitted at the Hospital for Tropical Diseases in Bangkok. For all patients the liver size was measured as number of centimeters below the rib cage, a stool examination was conducted, and CD23 and reactive nitrogen intermediates were measured. The median liver size was smaller in helminth-infected patients than in helminth-free patients (χ^2 for trend = 9.1, p = 0.003). Liver size significantly increased with the concentration of sCD23 ($p < 0.0001$). The median sCD23 concentration (OD) was significantly lower in helminth-infected patients than in helminth-free patients, respectively 0.33 (quartiles 0.24-0.57) and 0.45 (quartiles 0.27-0.59), (p = 0.01). There was a negative correlation between sCD23 concentrations and RNI (Spearman's rho = -0.40, $p < 0.0001$). All the above results remained significant after controlling for potential confounders. These results are compatible with a CD23/NO –mediated decrease in liver size in helminth-infected patients.

INTRODUCTION

Liver enlargement is a common feature of malaria (Manson *et al*, 1996). Its pathophysiology, however, is not clear. Erythrocytic and may be preerythrocytic parasite multiplication are thought to lead to mononuclear proliferation and non-specific hepatitis (Ramachandran and Perera, 1976). Intrahepatic sequestration of parasitized red blood cells may also contribute to hepatomegaly.

Nitric oxide (NO) is a key mediator of anti-malarial immunity (Anstey *et al*, 1996) but also has antiproliferative properties (Taylor-Robinson and Smith, 1999). Its generation requires the induction of the inducible NO synthase (iNOS), which can be achieved by a variety of immune mediators (Bogdan, 2001). Among these, the $Fc_{\varepsilon}R_{II}$ / CD23 receptor, upon ligation, can generate large quantities of NO (Dugas *et al*, 1995). In the absence of ligands, CD23 is normally physiologically cleaved into soluble CD23 (sCD23) (Delespesse *et al*, 1991), which has pleiotropic properties and is measurable in the plasma. On the contrary, upon ligation, the membrane CD23 receptor is stabilized, and cleavage is reduced (Pritchard *et al*, 1993). Recently, it was shown that helminth-infections were associated with protection from cerebral malaria. Adjustments for socioeconomic, nutritional factors and malaria history did not alter this association (Nacher *et al*, 2001a). It was suggested that the CD23/NO pathway had a protective role against cytoadherence and severe complications of malaria (Nacher *et al*, 2000; 2002). It was shown in Thailand that helminth-infected patients had a lower incidence of renal failure and jaundice during malaria (Nacher *et al*, 2001b), and that helminth-infected had higher reactive nitrogen intermediates concentrations (RNI), which correlated with IgE and sCD23 (Nacher *et al*, 2002). Here, the objective was to look for a possible influence of helminths and the CD23/NO pathway on liver size.

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MATERIAL AND METHODS

Between 1998 and 2000 a cross-sectional study was performed on 438 patients with confirmed *P. falciparum* malaria admitted to the Hospital for Tropical Diseases in Bangkok. Patients were recruited from the Intensive Care Unit and two general wards. After informed consent, a blood sample was taken from 288 patients on admission and frozen at -40ºC for further analyses. Patients had a stool examination for intestinal parasites (formalin-ether concentration technique). All intestinal helminth infections were grouped into one variable: presence of helminths. RNI were measured using the Griess method, and sCD23 was measured using ELISA (Pharmingen). As others (Ramachandran and Perera, 1976; Sowunmi, 1996), we considered the measure of a palpable liver (in centimeters below the rib line) as a reflection of liver enlargement. Adjustments for potential confounders including age, sex, body mass index, severity, duration of symptoms before admission, asexual parasitemia, schizontemia, total bilirubin, liver enzymes, were performed using the ordinal logit function (STATA®, College Station, Texas). Severity was represented in the model as 3 indicative variables, (<200,000/μl with no severe malaria criteria, ≥200,000/μl with no severe malaria criteria, and severe malaria according to WHO (2000).

RESULTS

There were 57 severe malaria patients, 210 patients with intermediate malaria, and 171 with mild malaria. The mean age was 26 (± 11) years. There were 321 men and 117 women. When compared to mild malaria, patients with severe malaria were more likely to have increased liver size, adjusted odds ratio (AOR) = 6 (95% CI = 2.6-9.1), p <0.001, so were those with hyperparasitemia but to a lesser degree AOR = 1.6, (95% CI = 1.001-2.6), p = 0.04. Patients with peripheral schizonts were twice more likely to have increased liver size AOR = 2, (95% CI = 1.05-3.8), p = 0.03.

The median liver size was smaller in helminth-infected patients than in helminth-free patients (χ^2 for trend = 9.1, p=0.003) (Table 1). This remained significant after adjustments for the potential confounders mentioned above (p = 0.01).

Liver size significantly increased with the concentration of sCD23 (p < 0.0001, see Table 1). This was still significant after adjusting for potential confounders mentioned above, and RNI, AOR =15.3, 95% confidence interval (CI) = 4.5-51.5, p <0.0001). On bivariate analysis liver size was inversely proportional to RNI concentrations (p = 0.009), however, this negative correlation between disappearance (p=0.5) when the sCD23 concentration was included in the model. Men

Table1
Relation between liver size and sCD23.

Liver size in cm (n1/n2)[a]	Helminths[b] (n)	No helminths[b] (n)	Median RNI concentration[c] [Quartiles] (OD)	Median sCD23 concentration[d] [Quartiles] (OD)
0 (299/193)	158	141	0.25 [0.16-0.4]	0.32 [0.23-0.52]
1 (35/23)	14	21	0.23 [0.15-0.38]	0.47 [0.34-0.56]
2 (57/35)	23	34	0.19 [0.16-0.28]	0.48 [0.33-0.61]
3 (20/17)	8	12	0.25 [0.15-0.4]	0.54 [0.38-0.79]
≥4 (27/20)	8	19	0.14 [0.12-0.18]	0.65 [0.53-0.82]

[a]n1 represents the number of files for which clinical and parasitological data was available, and n2 represents the number of files for which RNI and sCD23 were measured.
[b]Linear trend of odds between presence of helminths and liver size when using all 438 observations, p=0.003, when using 288 observations for which RNI and sCD23 were measured, p=0.02.
[c]Odds ratio (OR) = 0.15, (95% CI=0.03-0.7), p=0.009, but no longer significant in the adjusted model (ordinal logit regression), AOR = 0.6, (95% CI=0.1-3), p=0.5.
[d]Odds ratio = 21.5, (95% CI=7.5-61.5), p<0.0001, AOR = 12.8 (3.8-48), p<0.0001 (ordinal logit regression).

were more likely of having increased liver size (AOR = 2.3, 95% CI = 1.3-4, p=0.003. There was no relation between SGOT, SGPT, bilirubin and sCD23 (data not shown).

The median sCD23 concentration (OD) was significantly lower in helminth-infected patients than in helminth-free patients, respectively 0.33 (quartiles 0.24-0.57) and 0.45 (quartiles 0.27-0.59), (p=0.01). This remained significant after controlling for potential confounders using non-parametric regression (p=0.02). There was a negative correlation between sCD23 concentrations and RNI (Spearman's rho = -0.40, $p < 0.0001$). Both the negative correlation between sCD23 concentrations and RNI and the negative correlation between sCD23 and liver size remained independently significant after adjustments for all potential confounders ($p < 0.0001$ for both). As previously reported from a different dataset (Nacher *et al*, 2002), RNI concentrations (OD) were significantly higher in helminth-infected patients than in patients without helminths respectively 0.29 (quartiles 0.18-0.43) and 0.20 (quartiles 0.14-0.32), (p=0.0001). This remained significant after controlling for potential confounders using non-parametric regression (p=0.007).

DISCUSSION

After controlling for potential confounders, helminth-infected patients were thus less likely to have a palpable liver during falciparum malaria than helminth-free individuals. The very strong correlation between increased liver size and sCD23 concentrations and the lower sCD23 concentrations in helminth-infected patients suggest this molecule was related to the pathophysiology of liver enlargement during malaria. First, in the perspective where increased sCD23 is a consequence of hepatomegaly, increased sCD23 and liver size could have followed global mononuclear activation, leading to hepatic congestion and increased CD23 expression. Another way of interpreting these results, would be that the positive correlation between liver size and sCD23 reflected the role of the CD23/NO pathway in reducing liver congestion, either by reducing sequestration (Nacher *et al*, 2001b) either by reducing the proliferation of hepatic mononuclear cells, or both. High sCD23 levels in helminth-free patients might also have led to macrophage activation (Lecoanet-Henchoz *el al*, 1995) and subsequent liver congestion. The association between decreased liver size and helminths may thus have been linked to the chronic availability of IgE in helminth-infected patients, leading to CD23 stimulation thereby explaining the increased RNI concentrations and the lower sCD23 concentration in helminth-infected patients relative to helminth-free patients. However, after adjustments, RNI did not relate to liver size. The relatively low optical densities in our RNI assay may have resulted in insufficient power. Further studies, should explore the underlying mechanisms for these observations.

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